SYNTHESIS OF 2-VINYL HETEROCYCLES AS POTENTIAL ELECTROPHILIC COMPONENTS FOR INHIBITORS OF CRUZAIN AND NMR SPECTROSCOPIC ANALYSIS OF THEIR THIA-MICHAEL REACTIONS WITH THIOLS.

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ABSTRACT

Synthesis of 2-Vinyl Heterocycles as Potential Electrophilic Components for Inhibitors of Cruzain and NMR Spectroscopic Analysis of Their Thia-Michael Reactions with Thiols.

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Cruzain is an essential cysteine protease in *Trypanosoma cruzi* (*T. cruzi*), the causative agent of Chagas' disease.[1] Cruzain is an ideal target for development of potential trypanocidal drugs because of its critical role in the survival of T. cruzi in infected human hosts. Crystallographic analysis of cruzain treated with the covalent inactivator K11777, a peptidomimetic compound with an electrophilic vinyl sulfone substituent, undergoes the formation of a stable C-S bond between cruzain and the vinyl group of the inactivator. We seek to develop new cruzain inactivators in which the vinyl-sulfone group is replaced by isoelectronic vinyl--heterocycle groups which may provide reversible covalent inactivation of cruzain, which would be less subject to selectivity and toxicity problems that plague irreversible inactivators. This report examines the potential of 2-vinyl heterocycles as inhibitors for cruzain by NMR analysis of the thia-Michael addition to the vinyl bond using β-mercaptoethanol (BME), cysteamine (MEA) and L-glutathione (GSH) as Michael donors.

ACKNOWLEDGEMENTS

I would like to thank Dr. Meek, Bala and the rest of the lab members, for their guidance and support throughout the course of this research.

NOMENCLATURE

4-VP	4-VinylPyridine
2-VP	2-VinylPyridine
BME	β-mercaptoethanol
MEA	Cysteamine
GSH	L-glutathione
T. Cruzi	*Trypanosoma cruzi*

CHAPTER I

INTRODUCTION

Proposal

My research goal is to synthesize and explore the suitability of novel vinyl-heterocyclic compounds as reversible Michael acceptors to be used in the development of new inhibitors of cysteine proteases of therapeutic interest. The vinyl heterocycle compounds to be synthesized in which electron-withdrawing substituents are found on the heterocycle ring, and which are isoelectronic with normal Michael acceptors, and determine their utility in thia-Michael addition by thiols and thiolates, specifically mercaptoethanol, L-glutathione and cysteamine, using NMR spectroscopic analysis.

Introduction.

The triatomine bugs defecates on human host skin while it feeds on blood meal from its human host. The protozoan parasite *Trypanosoma cruzi* is found in the feces of the bug, therefore the infection occurs when feces are rubbed into the mucous membrane (e.g. eyes) or the bite wound.[2] *T. cruzi* enters host cells near the wound site in its trypanomastigote, or bloodstream, form. Once in the cell the protozoan parasite transforms into amastigotes, the immobile form of the pathogen.

After, the amastigotes divide, they transform into the mobile pathogen, the trypanomastigote form, which then bursts out of the cell and enters the bloodstream. Thus, the new trypomastigotes can infect other cells and repeat this lifecycle[3]. During the acute phase of disease, the infected human host either is asymptomatic or manifests a mild illness such as swelling at the site of the infection. When Chagas's disease advances into the chronic phase it can become deadly, resulting in heart failure, gastrointestinal problems, and increased risk of

stroke. A total of approximately 12 million people in Latin America and 300,000 in the United States are infected with Chagas's disease[4]. Current treatments face toxicity problems: chemotherapy with K11777 (clinical trials in humans are about to begin), nifurtimox, or benznidazole are undesirable because of the severe side effects. The drug K11777 targets the essential cysteine protease cruzain by forming a stable covalent bond with the vinyl sulfone substituent.[5] However, irreversible inactivators have limitations such as selectivity for target cysteine proteases and toxicity.[6]

In addition, the consensus mechanism for cysteine proteases involves nucleophilic attack by the cysteine thiol on the scissile peptide bond. As shown in **Figure 1**, in the enzyme's active site the thiol is deprotonated by the nitrogen in the histidine residue (Schneck et al. 2008; Zhai and Meek, manuscript in preparation). Once deprotonated, the thiolate undergoes nucleophilic attack to form an enzyme thioester with concomitant release of the amine product. Thus, the histidine residue is restored to its original deprotonated form and an intermediate with the cysteine thiol link to the thioester is formed. In the de-acylation half-reaction water, de-protonated by the thioester, hydrolyzes the thioester bond to form the carboxylate product, restoring the free enzyme.[7]

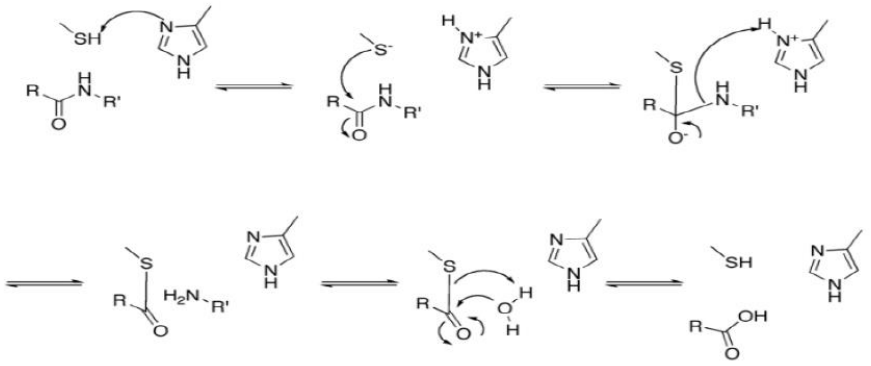


Figure 1. General chemical mechanism of the cysteine proteases.[7]

Thus, we synthesized 2-vinyl heterocycles with different electron-withdrawing and donating groups on the heterocycles in hopes of increasing the reactivity with the active site of cysteine of cruzain. We will use proton NMR analysis to determine if reversible covalent bonds are formed between added thiols and the vinyl group.

CHAPTER II

METHODS

All air and moisture sensitive reactions were carried out in oven-dried glassware under a nitrogen atmosphere with anhydrous solvents. All commercial reagents were obtained from commercial available sources and used without further purification. Proton NMR spectra of all reaction mixtures were performed on a 400 Hz Bruker AVANCE III HD NMR spectrometer.

*Synthesis of Boc-Phe-CO-N(OCH$_3$)CH$_3$ (**2**).* To a solution of t-Boc-L-phenylalanine (5.03 g, 18.8 mmol) in anhydrous DCM (100 mL), triethylamine (8 mL, 56 mmol, 3 eq),was added 1-propanephosphonic acid cyclic anhydride (18 mL, 28.2 mmol, 1.5 eq). The reaction mixture was placed in an ice bath and N,O-dimethylhydroxylamine hydrochloride was added (2.76 g, 28.2 mmol, 1.5 eq). The resulting yellow murky reaction mixture was stirred for 30 minutes. The reaction mixture was extracted with dichloromethane (DCM;100 mL, 3X), and then washed with water and brine. The combined organic layer was dried with Na_2SO_4, and concentrated under reduced pressure to afford a yellow oil (5.55 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19-7.14 (m, 5H), 4.92 (t, J=6.6 Hz, 1H), 3.66 (s, 1H), 3.44 (d, J=11.5 Hz, 1H), 2.71 (s, 2H), 1.32 (s, 9H). MS (ES) *m/z* 209.46 (M – H), R_t= 4.83 min.

*Synthesis of Boc-Phe-COH (**3**).* Boc-Phe-CO-N(OCH3)CH3 was dissolved in anhydrous THF (55 mL), and placed in an ice bath containing NaCl. To this clear solution, $LiAlH_4$ (11.25 mL, 22.3 mmol, 1.25 eq) was added dropwise, and the resultant reaction mixture was stirred for 1 h. The reaction mixture was quenched at 0℃ by adding 5% HCl in H_2O and a white precipitate formed. The resulting mixture was neutralized with sodium bicarbonate and concentrated under vacuum. The resulting white solid was diluted with H_2O, extracted with ethyl acetate (3 x 200 mL), and the organic layer was washed with brine. The combined organic layer was dried with

Na_2SO_4, filtered and concentrated to afford a light yellow oil (4.46 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.72 (s, 1H), 7.35-7.25 (m, 5H), 4.11 (t, J=7.7Hz, 1H), 3.12 (d, J=8 Hz, 2H), 1.48 (s, 1H).

*Synthesis of Boc-Phe-2-Vinyl-Pyridine (**5**).* To a solution of triphenylphosphonium 2-methylpyridine (4) (1.96 g, 4.57 mmol) in THF (50 mL) under nitrogen atmosphere was placed in an ethanol-dry ice bath (-70℃) added dropwise lithium bis(trimethylsilyl)amide (10 mL, 6.4 mmol, 2.2 eq). After stirring for 15 min at -70℃ Boc-Phe-H in 5 mL of THF was added dropwise and stirred at -70℃ for 1h. The reaction was quenched by adding glacial acetic acid (0.1 mL), and immediately sodium bicarbonate(30 mL). The reaction mixture was transferred into a separatory funnel. The water layer was extracted with ethyl acetate (2 x 200 mL), washed with brine and dried the combined organic layers on sodium sulfate. Filtered off the drying agent and filtrate was concentrated to get the crude product, which was purified by column chromatography (5%-20% ethyl acetate/hexane) to afford a clear oil (0.19 g, 18.8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.5 (d, J=4.3 Hz, 2H), 7.62 (t, J=6.41 Hz, 1H), 7.32-7.28 (m, 3H), 7.25-7.22 (m, 5H), 6.75-6.69 (m, 1H), 6.57 (d, J=15.8 Hz, 1H),4.34 (m, 1H), 3.04 (m, 2H), 1.32 (s, 3H). MS (ES) *m/z* 325.3 (M – H), R_t= 3.7 min.

*Synthesis of methyl methanesulfonate (**6**).* Into a three-neck round bottom flask was added sodium bicarbonate (6 g) and water (10 mL) to make a supersaturated solution, which was placed under an ice bath (9 ℃). To this solution dichloromethane (20 mL), triethylamine (0.27 mL, 1.94 mmol, 0.03 eq), methanol (6.3 mL, 155.04 mmol, 2.4 eq), and methanesulfonyl chloride (5 mL, 64.6 mmol) were added, and the reaction was stirred overnight under an ice bath. The subnatant organic layer was decanted and concentrated to afford the product methyl methanesulfonate (2.64 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H), 2.97 (s, 1H).[8]

Methylation of Boc-Phe-2-Vinyl-Pyridine (**7**). Methyl methanesulfonate (50.9 mg, 0.462 mmol) and Boc-Phe-Vinyl-Pyridine (100 mg, 0.31 mmol) were added into a vial with benzene (4 mL), sealed and stirred at room temperature for 2 weeks. However, NMR analysis showed that the vinyl proton peaks were absent.[9]

*Methylation of Triphenylphosphonium salt of 2-methylpyridine (**8**).* Into a mixture of ice (2.42 g) and sodium carbonate (0.0613 g), the phosphonium 2-methylpyridine (0.41 g, 0.94 mmol) was added and dissolved with ethyl acetate. The pH was checked to be basic (pH 8) and extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate and concentrated. The resulting white precipitate was dissolved in benzene and methyl methanesulfonate (0.156 g, 1.4 mmol, 1.5 eq) was added, and the reaction mixture was stirred at room temperature for 2 weeks.[9] The reaction was unsuccessful.

*Synthesis of 2-(Boc-amino)pyrimidine (**9**).* To a solution (in an ice bath) of 2-aminopyrimidine (2.02 g, 21 mmol) in dichloromethane (30 mL), di-tert-butyl dicarbonate (6.65 g, 25.2 mmol, 1.2 eq), DMAP (0.28 g, 2.1 mmol), and triethylamine (4.4 mL, 31.5 mmol) were added under nitrogen atmosphere. The resulting yellow murky solution was stirred at room temperature for 2h. The reaction mixture was diluted with water, extracted with dichloromethane, washed with brine and the combined organic layers were dried with sodium sulfate and concentrated to afford an orange oil. The oil was purified by column chromatography (10%-25% ethyl acetate/hexane) to afford a clear oil (3.26 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (d, J= 4.8 Hz, 2H), 6.91 (t, J= 4.7 Hz, 1H), 1.43 (s, 3H).

*Synthesis of 4-vinyl-2-(Boc-amino)pyrimidine (**10**).* To a solution of 2-(t-Boc-amino)pyrimidine in THF (10 mL) in a Dry Ice/acetone bath, vinyl magnesium bromide was added dropwise. The resulting yellow solution was stirred for 2h. However, NMR analysis did not show new vinyl proton peaks.[10]

*1-Methyl-2-vinylpyridinium iodide (**11**).* To a solution of 2-vinylpyridine (700 mg, 6.66 mmol) in methanol (10 mL), methyl iodide (4.5 g, 31 mmol) was added and stirred for 48 h at room temperature. The solvent was removed under rotary evaporation and the solid washed three times with Et_2O. After drying under vacuum a dark green oil (400 mg), was recovered. ^{1}H NMR (400 MHz, D2O) δ 8.68 (d, J= 6.3 Hz, 2 H), 7.79 (d, J= 8.7 Hz, 2 H), 6.82-6.6.75 (dd, J_1= 11.2 Hz, J_2= 11.0 Hz, 1H),6.09 (d, J= 17.7 Hz,1 H), 5.54 (d, J=11.4 Hz, 1 H), 3.31 (s, 3 H)[11]; MS (ES) *m/z* 120.4 (M – H), R_t= 0.36min.

*1-Methyl-4-vinylpyridinium Iodide (**12**).* To a solution of 4-vinylpyridine (0.68 g, 6.5 mmol) in methanol (10 mL), methyl iodide (4.56 g, 32.1 mmol) was added and stirred for 48 h at room temperature. The solvent was evaporated and the resulting solid washed with Et_2O (4 × 20 mL) and CH_3OH (50 mL), dried in a vacuum, and recrystallized with EtOH-Et_2O to obtain a light yellow solid (1.39 g, 86.9%). ^{1}H NMR (400 MHz, D_2O) δ 8.60 (d, J= 6.7 Hz, 2 H), 7.89 (d, J= 6.1 Hz, 2 H), 6.95-6.88 (dd, J_1= 12.3 Hz, J_2= 10.7 Hz, 1H), 6.42 (d, J= 17.7 Hz, 1 H), 5.94 (d, J= 10.7 Hz, 1 H), 3.55 (s, 3 H) ; MS (ES) *m/z* 120.4 (M – H), R_t= 0.47min.[12]

*Methylation of compound **5** (**13**).* To a solution of compound **5** (96.1 m g, 0.296 mmol) in methanol (10 mL), methyl iodide (0.21 g, 1.48 mmol) was added and stirred for 48 h at room temperature. The solvent was evaporated and the resulting solid washed with Et_2O (4 × 20 mL) and CH_3OH (50 mL), dried in a vacuum, and recrystallized with EtOH-Et_2O to obtain a light yellow solid (64.4 mg, 64%). ^{1}H NMR (400 MHz, D_2O) δ 8.85 (d, J= 7.1 Hz, 1 H), 8.50 (t, J=

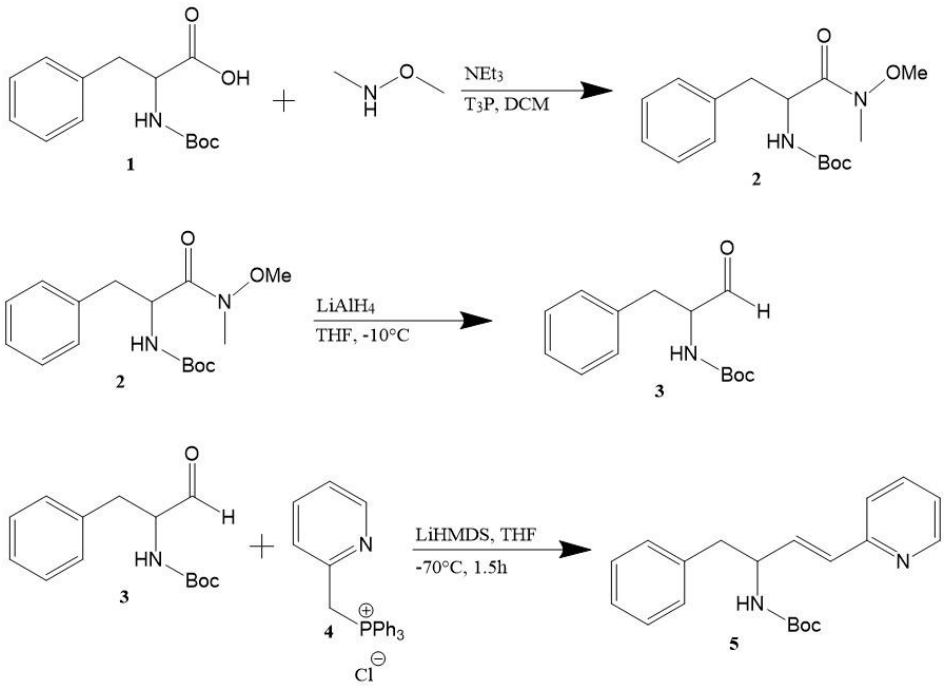
7.1 Hz, 1 H), 8.23 (d, J= 8.3 Hz, 1H), 7.92 (t, J= 7.1 Hz, 1H), 7.35-7.23 (m, 5H), 6.99-6.94 (m, 1H), 6.87 (d, J= 16.1 Hz, 1 H), 4.67 (m, 1 H), 4.25 (s, 3 H), 1.44 (s, 9 H); MS (ES) *m/z* 339.3 (M – H), R_t= 0.53min.[12]

*NMR Analysis of Michael Addition with Thiols***.** As an example of the thiolate addition studies, L-glutathione was mixed with compound **11** (4.1 mg, 0.034 mmol) dissolved in D_2O (0.5 mL) in a standard 5 mm NMR tube, and the spectrum was recorded (**Figure 5** blue spectrum). L-glutathione (27.4 mg, 0.089 mmol, 3 mol equiv) was then added, and the spectrum was recorded 1 day after the addition (**Figure 5** red spectrum).[13]

CHAPTER III

RESULTS/DISCUSSION

The t-Boc phenylalanine derivative containing the 2-vinyl pyridine (compound **5**) was the first compound in our studies to be synthesized, and it was examined for the thia-Michael addition to the vinyl bond by NMR analysis. Compound **5** was synthesized from Boc-L-phenylalanine (AK Scientific). As shown in scheme 1, the carboxylic acid group on the Boc-L-phenylalanine was converted to a Weinreb amide **2**, followed by its reduction to an aldehyde **3**.Then 2-vinyl pyridine (compound **5**) was prepared by the Wittig reaction of aldehyde **3** with the triphenylphosphonium ylide (**4**).



Scheme 1. Synthetic plan for making compound 5.

Once compound **5** was purified it was reacted with a Michael donor, 2-mercaptoethanol (BME), in DMSO-d_6/ D_2O (3:1) at 10:1 eq:eq to compound **5**. One drop of NaOD was added to

the thia-Michael reaction in order to deprotonate BME. The reaction was monitored hourly by proton NMR but no addition was detected. Even after 1 week BME did not attack the vinyl bond in compound **5**. Thus, having the nitrogen in the 2 position in the pyridine group is not the best position for thia-Michael additions. Using the same conditions for the thia-Michael addition reaction for compound **5**, the vinyl benzene derivative of t-Boc phenylalanine (compound **LL-26**; synthesized by a coworker, Linfeng Li, in the Meek lab) was monitored by proton NMR. Unsurprisingly, since there is insufficient electron withdrawal in the vinyl-benzene group, in comparison to the vinyl sulfone group in K11777, whereinthe thiolate performs a nucleophilic attack on the vinyl bond after being deprotonated by the histidine residue.

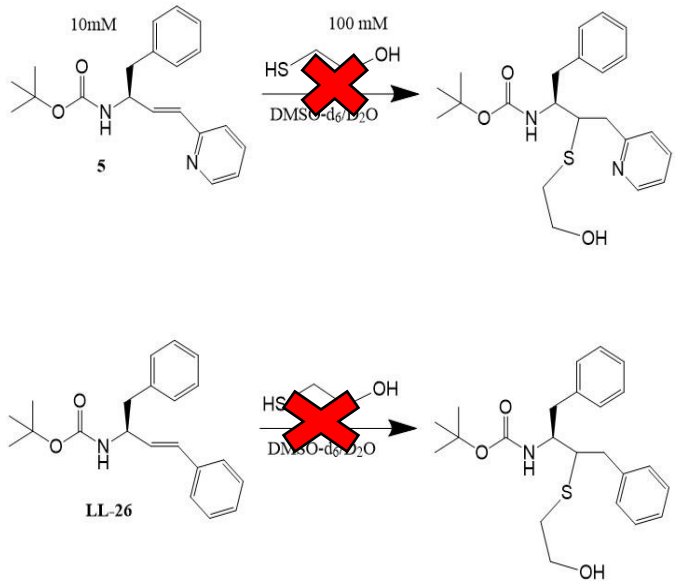


Figure 2. Thia-Michael addition reactions for compound **5** and **LL-26**. In an NMR tube 10 mM of compound **5** and 100 mM of BME + 1 drop of NaOD (pH 11) in 0.5 mL of DMSO-d_6/D_2O (3:1).

Next, compound **BC-90** (synthesized by a coworker, Bala Chenna, in the Meek lab) with two electron-withdrawing groups (4,6-dichloro) on the pyrimidine group was treated with BME

using the same conditions as for compound **5**. However, when proton NMR was taken the sample was too dilute to obtain an interpretable spectrum.

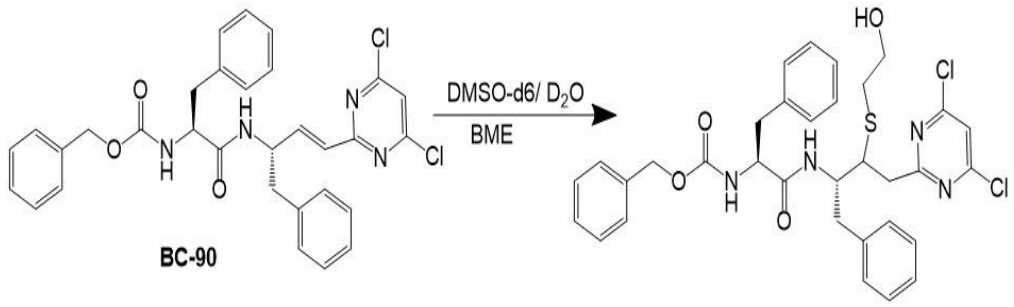


Figure 3. Thia-Michael addition reactions for compound **BC-90**.

As a result of having a limited amount of **BC-90**, 100 microliters of a 200 mM solution of **BC-90** was added to the NMR tube. NMR analysis was repeated and the vinyl proton peaks from the **BC-90** compound were observed (although the sample was not very concentrated) around 6 ppm. **Figure 4**, shows the NMR spectrum of the thia-Michael addition reaction with **BC-90** after zero minutes, shows two doublets (the vinyl protons) between 6.5 and 6.0 ppm. The red spectrum was recorded after 1 day. After one day the vinyl proton peaks were absent, indicating that thia-Michael addition from BME had occurred.

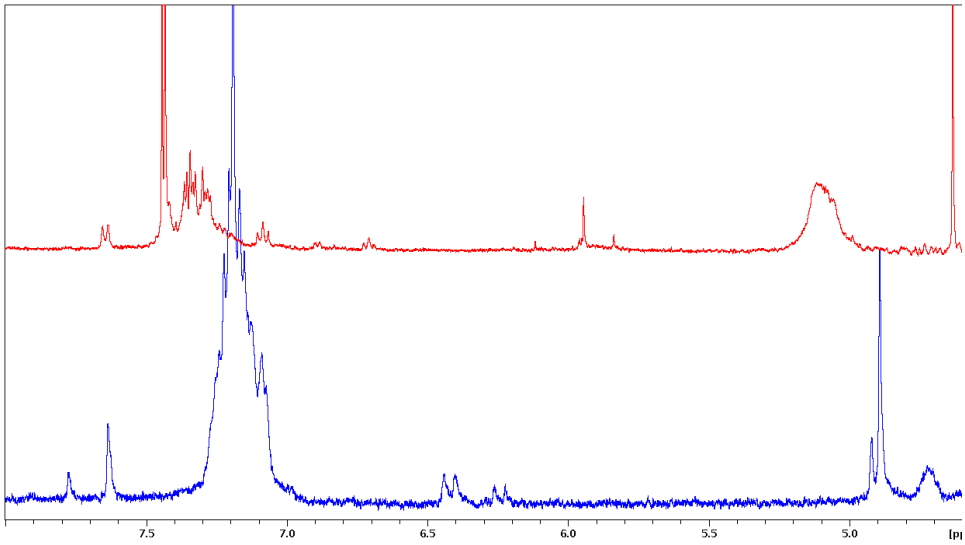


Figure 4. NMR spectrum from 8 ppm to 4 ppm of **BC-90.** The blue spectrum is of BC-90 in DMSO-d_6.The red spectrum shows the thia-Michael addition after 1 day.

Thirdly, it was hypothesized that if the nitrogen in the 2-position of the pyridine ring in compound **5** was methylated it would stabilize the negative charge arising from thiolate attack on the vinyl group. Thus, an attempt to prepare methylated compound **5** was done using methyl methanesulfonate dissolved in benzene. However, after two weeks compound **7** was not observed, but instead the vinyl proton peaks had disappeared.

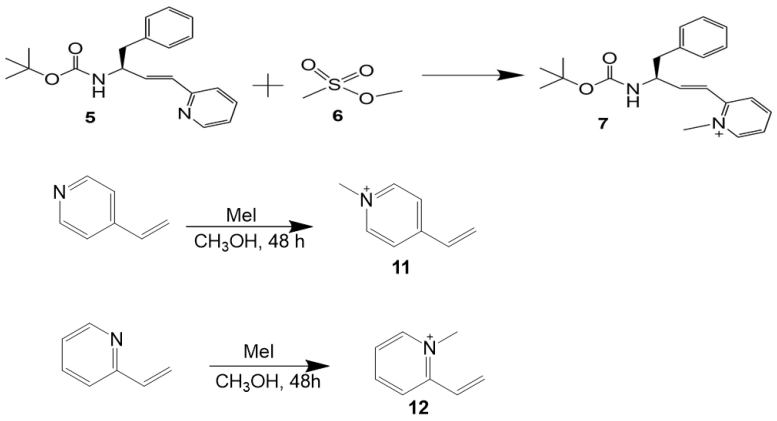


Figure 5. Methylation of compound **5**, **11**, and **12**.

Furthermore, since the protocol for the methylation that uses methyl methanesulfonate takes two weeks, has poor yields, and most importantly was not successful for compound **5,** another approach was taken[9]. Methylation of 4-vinylpyridine and 2-vinylpyridine was

completed by reaction with methyl iodide in methanol for 48 hours. Compounds **11** and **12** were characterized by mass spectrometry and proton NMR. The solvent was evaporated under pressure, however for higher purity, a recrystallization step should be done. In addition, Avonto and coworkers published a paper in Angewandte Chemie (ref) in which they developed a protocol to identify Michael acceptors, and test their reversibility by doing a dilution in deuterated chloroform[13]. As a result, a similar experiment to what they report was performed, but since the methylated compounds and L-glutathione are soluble in water, D_2O was used as the NMR solvent instead of DMSO-d_6. Two solutions were made with 64.4 mM of compounds **11** and **12** and 180 mM (3 eq) of L-glutathione in 0.5 mL of D_2O. After 5h vinyl proton peaks from both reactions were still observed, thus the reaction mixtures were left for 1 day and the spectrums was the recorded. After 1 day both reactions mixture for compounds **11** and **12** showed addition of mercaptoethanol to the vinyl group. In **Figure 6**, the blue spectrum is of the pure compounds **11** and the green spectrum is of the pure compound **12**. The spectrums for compounds **11** and **12** both show a doublet of doublets near 6.80 ppm and two doublets around 6.10 and 5.50 ppm. The red (**11**) and purple (**12**) spectrum show the vinyl proton peaks of compounds **11** and **12** were completely eradicated by treatment with mercaptoethanol.

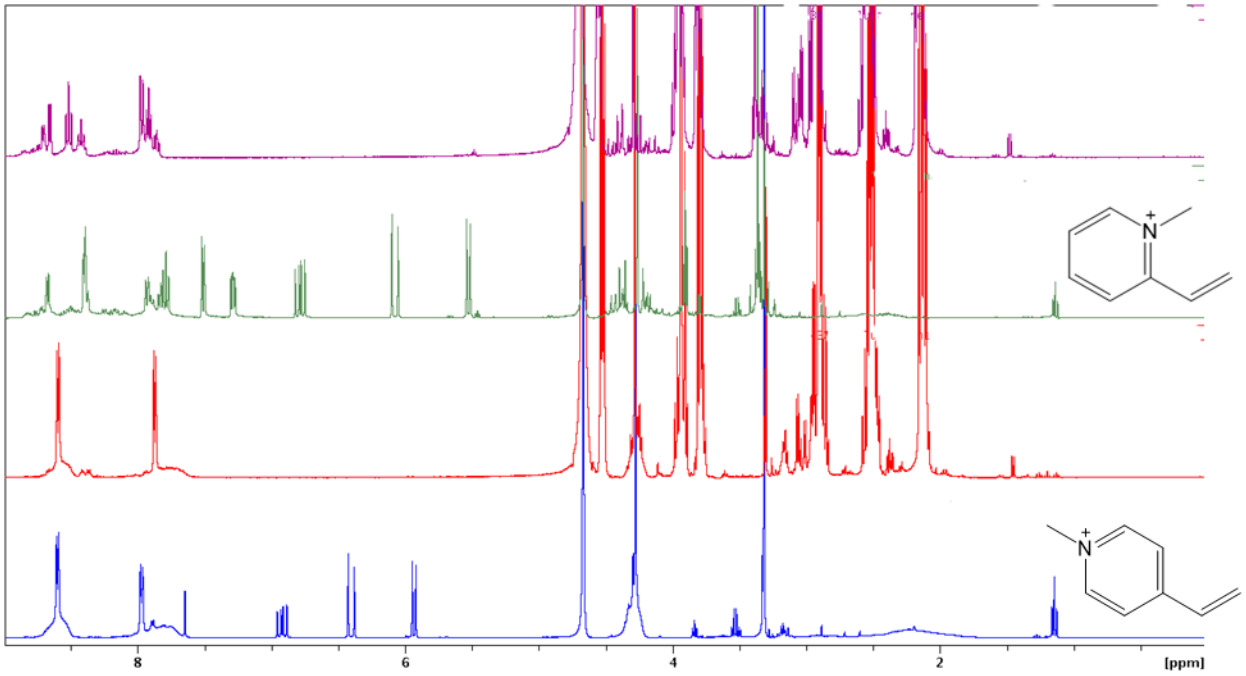


Figure 6. NMR spectrum from 0-9 ppm of compound **11** and **12**. The blue spectrum is 64.4 mM compound **11** in DMSO-d_6. The red spectrum contains 64.4 mM mM compound **11** and 180 mM mM mercaptoethanol, and the absence of the vinyl proton peaks between 5.8-6.4 ppm indicates full thia-Michael addition of compound **11** by BME after 1 day. The green spectrum is 64.4 mM compound **12** in DMSO-d_6. The purple spectrum show the thia-Michael addition of 180 mM mM mercaptoethanol to compound **12** after 1 day.

Furthermore, the thia-Michael addition studies was performed with 4-vinylpyridine and 2-vinylpyridine using 2-mercaptoethanol (BME), cysteamine, and L-glutathione as Michael donors. These vinyl-pyridines are models for the incorporation of the vinyl-pyridine substituent into peptidomimetics. As expected 4-vinylpyridine showed addition while 2-vinylpyridine, while did not, even after 2 days of incubation. Thus, compound **5** was also N-methylated and reacted with L-glutathione using the same conditions for the thia-Michael addition of compounds **11** and **12.**

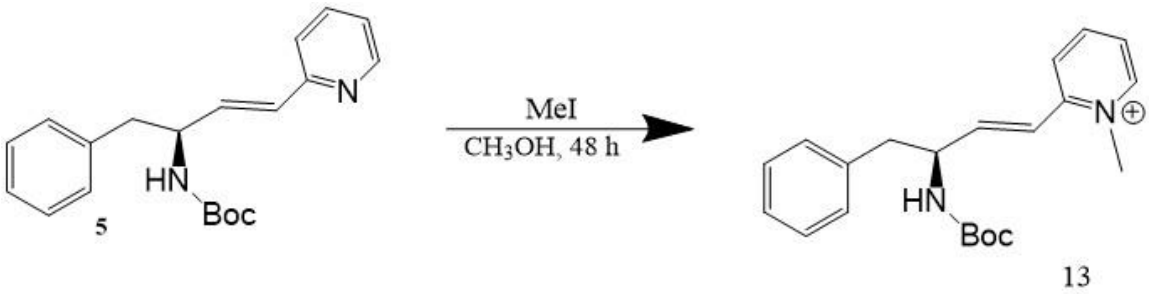


Figure 7. Methylation of compound **5** with methyl iodide in methanol for 48 h.

Thus, compound **5** was methylated with methyl iodide in methanol for 48 hours, shown in **Figure 7** . Compound **5** was characterized by mass spectrometry and proton NMR. The solvent was evaporated under pressure to afford a yellow powder. After, thia-Michael addition was performed by dissolving compound 5 (112 M) in 0.5 mL of DMSO- d_6/ d_2O (3:1) and adding BME (1.12M) and NaOD (10 µL). In approximately 5 minutes, the nucleophilic sulfur in BME had added to the vinyl group because the vinyl peaks were absent. Studies done with cruzain shows inhibition with compound **13 (**with a cbz protecting group instead of Boc), but the potency of inhibition is weak (Ki^* <10 µM).

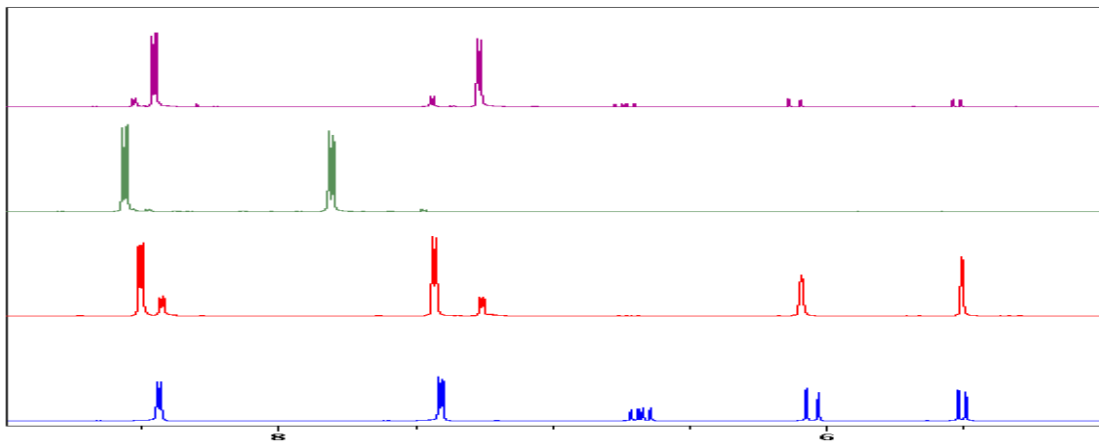
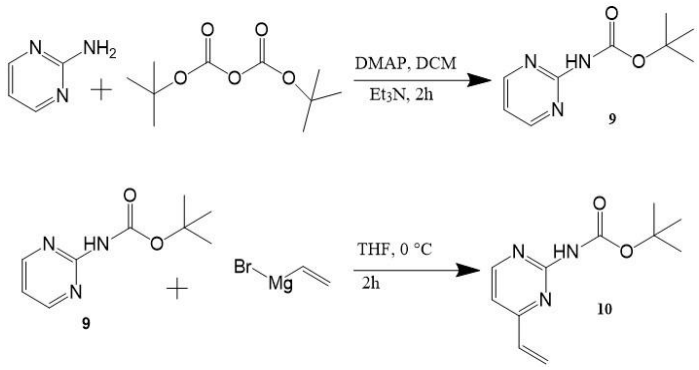


Figure 8. NMR spectra from 5-9 ppm of 4-vinylpyridine with 2-mercaptoethanol (BME) heated at 40 degrees C for 90 minutes. 1) blue NMR spectrum is of pure 192 mM 4-vinylpyridine in 0.5

mL DMSO-d6. 2) red NMR spectrum is of 192 mM 4-vinylpyridine and 383 mM BME in 0.5 mL DMSO-d6. 3) Green NMR spectrum is of 192 mM 4-vinylpyridine and 383 mM L-glutathione in 0.5 mL D20. 4) Purple NMR spectrum is of 192 mM 4-vinylpyridine and 383 mM cysteamine in 0.5 mL DMSO-d6. Upon adduct formation the pyridine protons' chemical shift move slightly downfield.

Compound 5 was reacted with cysteamine in DMSO-d6 and heated at 40 °C for 3 hours. However, cysteamine did not attack the vinyl bond since the vinyl proton peaks remained visible at 3 hours and after 5 days. In addition, 4-vinylpyridine was reacted with BME, cysteamine, L-glutathione, and heated (40 °C) to catalyze the reaction, shown in Figure 8. The spectrum of the reaction was recorded at 30 min and 90 min. At 30 minutes vinyl peaks were still visible for all three Michael donors. After 90 minutes, the vinyl peaks were absent for the reaction mixtures with L-glutathione and BME, but with treatment with cysteamine, traces of the vinyl proton peaks remained. Thus, successful thia-Michael addition reactions can be observed in 90 minutes if samples are heated, in lieu of a day at ambient temperature to observe addition. L-glutathione and BME are better Michael donors than cysteamine, however they each have their limitations. L-glutathione is soluble in water, slightly soluble in DMSO, and insoluble in chloroform, thus if the vinyl compound is insoluble in water BME is a better Michael donor. BME is miscible in water, DMSO, and chloroform. However, in order for BME to be capable of Michael addition, about 10 uLs of NaOD must be added. Without the addition of base, traces of the vinyl proton peaks will be visible in NMR after 1 day of incubation. Also, when 3:1equivalents of BME: vinyl substrate were used, no addition was observed.Therefore BME must be used in a 10-fold equivalent ratio.

Lastly, we attempted to prepare compound **10** from 2-aminopyrimidine. First, the amino group was protected using di-tert-butyl dicarbonate to produce compound **9**. Next, a Grignard reaction was performed using compound **9** and vinyl magnesium bromide in THF. However, the reaction was not successful since no vinyl peaks were observed in NMR.



Scheme 2. Synthetic plan for making compound **10**.

However, mass spectrometry analysis of a 10-microliter sample of the crude compound **10** indicated that the t-Boc had been removed. Thus, a nominal amount of compound 1**0** was made but not enough to be detected by NMR analysis.

CHAPTER IV

CONCLUSION

Synthesis of compound **5** was successful however, NMR spectroscopic analysis of its thia-Michael reactions with thiols showed that no covalent bond was formed between the thiolate and the vinyl β-carbon. Yet, methylation of the nitrogen in 4-vinylpyridine and 2-vinylpyridine resulting in analogues that fostered thia-Michael addition. Thus, thia-Michael addition to compound **5** was achieved when the pyridine nitrogen is N-methylated. However, the inhibition of compound 13 on cruzain was not potent enough. Thus, more heterocycles with electron-withdrawing groups such as compound **BC-90,** which shows potential for Michael addition, should be explored. Lastly, heating the thia-Michael reaction mixtures will catalyze the addition of the thiolate to the vinyl-pyridine in 2 hours using L-glutathione and BME as Michael donors.

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APPENDIX

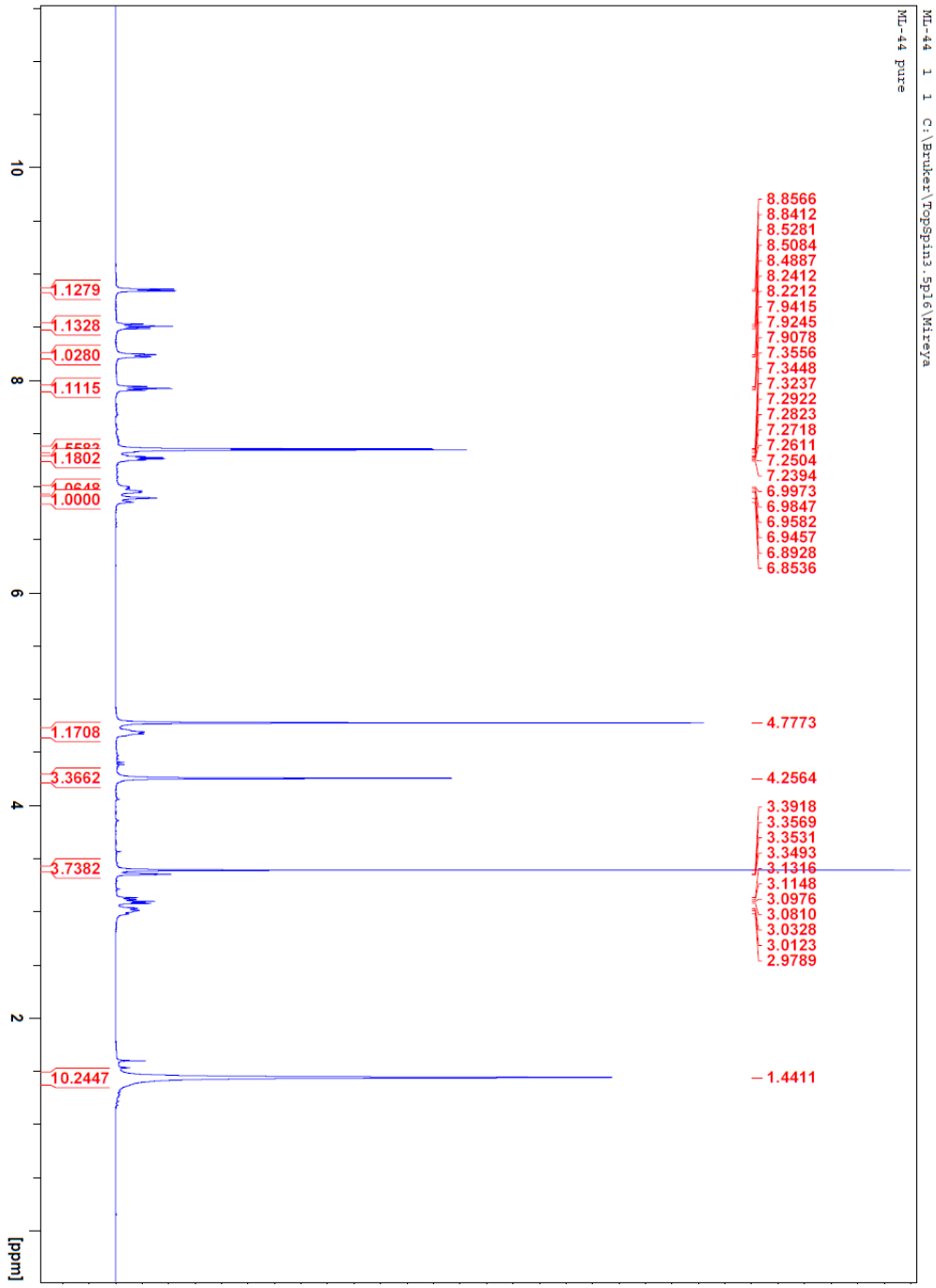

Figure 9. Proton NMR of compound **13** in D_2O.

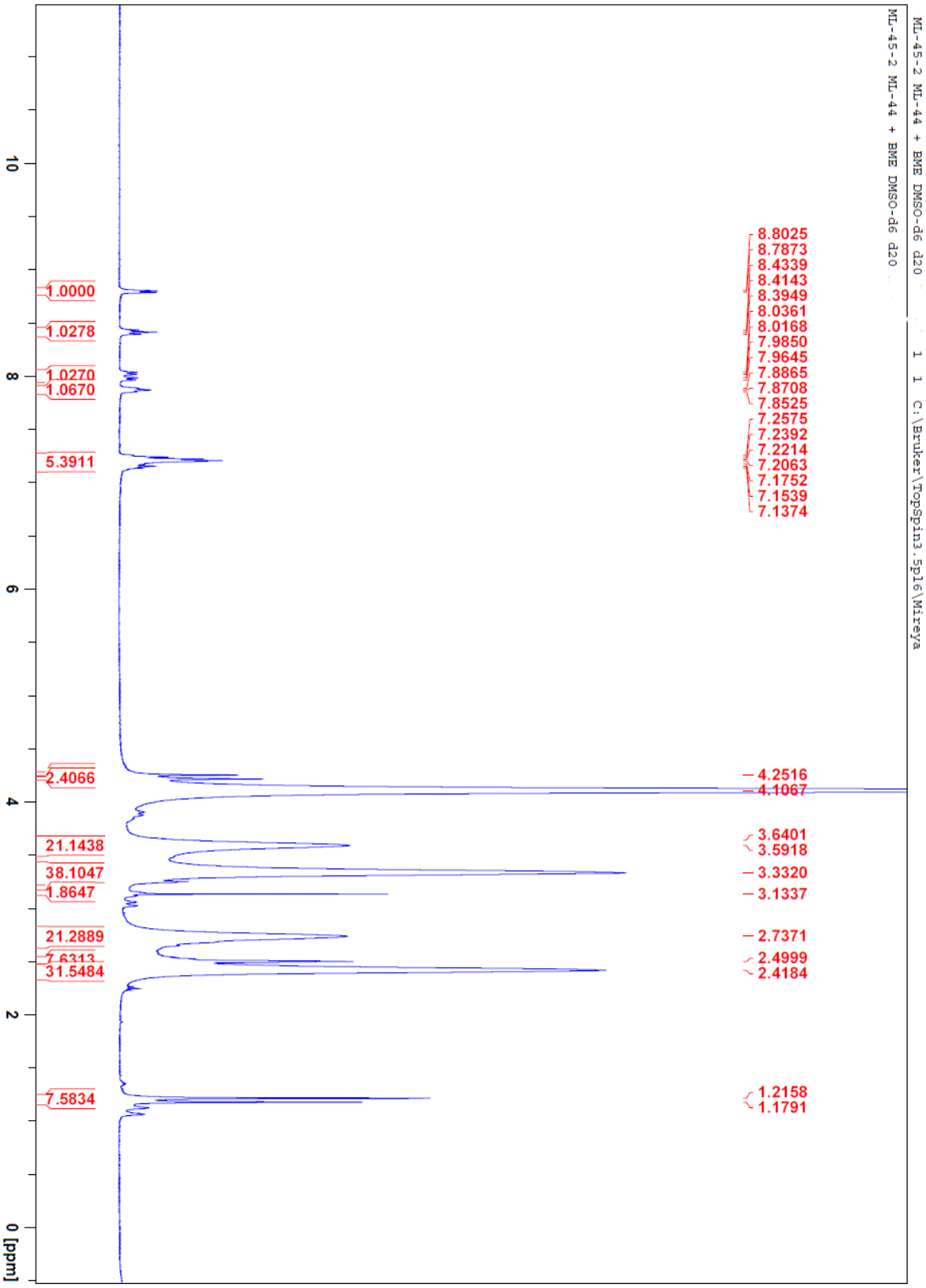

Figure 10. Proton NMR of thial-michael addition of compound **13** with BME in DMSO-d6/D2O.